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Identical Hik-Rre Systems Are Involved in Perception and Transduction of Salt Signals and Hyperosmotic Signals but Regulate the Expression of Individual Genes to Different Extents in *Synechocystis**

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In previous studies, we characterized five histidine kinases (Hiks) and the cognate response regulators (Rres) that control the expression of ~70% of the hyperosmotic stress-inducible genes in the cyanobacterium *Synechocystis* sp. PCC 6803. In the present study, we screened a gene knock-out library of Rres by RNA slot-blot hybridization and with a genome-wide DNA microarray and identified three Hik-Rre systems, namely, Hik33-Rre31, Hik10-Rre3, and Hik16-Hik41-Rre17, as well as another system that included Rre1, that were involved in perception of salt stress and transduction of the signal. We found that these Hik-Rre systems were identical to those that were involved in perception and transduction of the hyperosmotic stress signal. We compared the induction factors of the salt stress- and hyperosmotic stress-inducible genes that are located downstream of each system and found that these genes responded to the two kinds of stress to different respective extents. In addition, the Hik33-Rre31 system regulated the expression of genes that were specifically induced by hyperosmotic stress, whereas the system that included Rre1 regulated the expression of one or two genes that were specifically induced either by salt stress or by hyperosmotic stress. Our observations suggest that the perception of salt and hyperosmotic stress by the Hik-Rre systems is complex and that salt stress and hyperosmotic stress are perceived as distinct signals by the Hik-Rre systems.

Responses to salt stress and hyperosmotic stress have been investigated in prokaryotes, fungi, and plants. However, there is some confusion in the literature because salt stress and hyperosmotic stress have been regarded both as equivalent and as distinct stimuli (1–4). In *Arabidopsis thaliana*, both salt stress due to 0.1 M NaCl and hyperosmotic stress due to 0.2 M mannitol regulate the expression of not only the same set of genes but also of different sets of genes (4). In the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter, *Synechocystis*), it is clear that there are major differences between the sets of genes that respond to salt stress due to 0.5 M NaCl and hyperosmotic stress due to 0.5 M sorbitol (3). Moreover, the cytoplasmic volume of *Synechocystis* decreases by ~70% of the original volume within 10 min when cells are exposed to 0.5 M sorbitol, but the decrease in cytoplasmic volume is only 30% with 0.5 M NaCl (3). Although the responses to hyperosmotic stress and salt stress are different in terms of gene expression and changes in cytoplasmic volume, recent studies have demonstrated that the same histidine kinases (Hiks),[1] such as Hik33, Hik34, and Hik16, might be involved in the perception of salt and hyperosmotic stress (5, 6).

In *Synechocystis*, several Hiks that are paired with specific response regulators (Rres) have been identified as regulators of the response to hyperosmotic stress (6). A specific Hik senses hyperosmotic stress, and it seems likely that the signal is transferred to the cognate Rre by transfer of a phosphate group from the histidine residue of the Hik to an aspartate residue in the receiver domain of the Rre, which in turn acts either to derepress or to induce the expression of downstream genes. Screenings using yeast two-hybrid systems (7) have also provided evidence for the physical interactions between respective members of cognate pairs of Hiks and Rres.

The genome of *Synechocystis* encodes 47 Hiks and 45 Rres (Refs. 8 and 9; see also www.kazusa.or.jp/cyanobase/Synechocystis/index.html). We have constructed libraries of knock-out mutants of these genes as part of a program aimed at elucidating the specific combinations of Hiks and Rres that are associated with the perception and transduction of a variety of stress signals. A previous study demonstrated that Hik33, which was identified first as a cold sensor (10), is also involved in perception of hyperosmotic stress (11). Studies involving systematic mutagenesis of Hiks and Rres in conjunction with DNA microarray analysis have demonstrated that Hik34, Hik10, and a combination of Hik16 plus Hik41 are also involved in perception of hyperosmotic stress. We have identified the Rres located downstream of the Hiks in the pathway for transduction of the hyperosmotic stress sig-

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[1] The abbreviations used are: Hik, histidine kinase; Rre, response regulator; ORF, open reading frame; RE, effective ratio.

nal (6). The specific cognate partners in the sensing of hyperosmotic stress are Hik33-Rre31, Hik10-Rre3, Hik34-Rre1, Hik16-Hik41-Rre17, and possibly Hik2-Rre1.

The histidine kinases Hik33, Hik34, Hik16, and Hik41 have also been identified as components of salt signal-sensing and transducing systems (11). However, we do not know how many salt-responsive genes they might regulate, and the specific Rre associated with each Hik remains to be identified. It is possible that other Hiks might be involved in the sensing of salt stress that have not yet been discovered because of our limited understanding of the way the entire genome responds to salt stress. The responses of the entire genome can be monitored by DNA microarray analysis. A better understanding of the responsiveness of each gene to mutations in specific Hiks would allow us to identify the Hik that is associated with each stress-responsive gene and to identify genes that are not responsive to Hiks that have already been identified and must, thus, be controlled by unidentified factors.

In the present study, we identified Hik10 and Hik2 as possibly novel salt-sensing Hiks, as well as the cognate Rres of Hik33, Hik34, Hik16, and Hik41. We compared salt-induced genes with hyperosmotic stress-induced genes and found that the two kinds of stress induced the expression of individual genes to different extents. Our findings demonstrate that the individual systems for recognition of salt stress and hyperosmotic stress are shared, but the two kinds of stress are perceived as different signals.

EXPERIMENTAL PROCEDURES

Strains and Culture Conditions—Synechocystis sp. PCC 6803, a glucose-tolerant stain, was kindly provided by Dr. J. G. K. Williams (E. I. du Pont de Nemours & Co., Wilmington, DE), and a glucose-sensitive strain was obtained from Professor S. Shestakov (Department of Genetics, Moscow State University, Russia). These two strains served as wild-type strains for construction of the gene knock-out libraries of Hiks and Rres, as described previously (Refs. 6, 12; see also www.kazusa.or.jp/cyanobase/Synechocystis/mutants/).

Wild-type cells were grown photoautotrophically at 34 °C in 50 ml of BG-11 medium buffered with 20 mM HEPES-NaOH (pH 7.5) under continuous illumination from incandescent lamps at 70 microeinstein $m^{-2}s^{-1}$, with aeration by air that contained 1% CO_2, as described previously (13). Mutant cells were grown under the same conditions as wild-type cells except in the case of precultures, in which BG-11 medium was supplemented with an antibiotic (20 μg ml^{-1} spectinomycin or 25 μg ml^{-1} kanamycin for cells in which the genome had been mutated by insertion of a spectinomycin-resistance gene cassette or a kanamycin-resistance gene cassette, respectively). For exposure of cells to salt stress, a solution of 5.0 M NaCl was added to 50 ml of a suspension of cells that had been grown under standard conditions for 16 h, to give a final concentration of 0.5 M. The duration of incubation under salt stress was 20 min, the same duration and molar concentration (of sorbitol) used for the experiments with hyperosmotic stress.

Isolation of RNA—After incubation of cultures under designated conditions, 50-ml aliquots were rapidly combined with an equal volume of ice-cold ethanol that contained 5% (w/v) phenol for instantaneous killing of cells and to prevent degradation of mRNA. After collection of the killed cells by centrifugation at 1,000 × *g* for 5 min at 4 °C, total RNA was isolated by the hot phenol method as described previously (14). The extracted RNA was treated with DNase I (Nippon Gene, Tokyo, Japan) to remove contaminating DNA and then purified with a mixture of phenol, chloroform, and isoamyl alcohol (25:24:1, v/v) and precipitated in ethanol.

RNA Slot-blot Hybridization and Northern Blotting—For RNA slot-blot hybridization (15), 10 μg of total RNA were applied to a Hybond-N^+ nylon membrane (Amersham Biosciences). The RNA on the membrane was allowed to hybridize with a specific probe that had been generated from a salt stress-inducible gene, such as *slr1544*, *dnaK2* (*sll0170*), *slr0967,* or *ndhR* (*sll1594*).

For Northern blotting, 15 μg of total RNA were fractionated by electrophoresis on a 1.2% agarose gel that contained 2.05 M formaldehyde. The RNA was transferred to a Hybond-N^+ nylon membrane by capillary transfer and allowed to hybridize with a specific probe. Labeling, hybridization, and washing were performed as described in instructions supplied with the AlkPhos Direct labeling and detection system with CDP-star (Amersham Biosciences). DNA probes were conjugated with alkaline phosphatase (Alkphos Direct kit). After hybridization and washing, blots were soaked in CDP-star solution (Amersham Biosciences), and signals from hybridized transcripts were detected with a luminescence image analyzer (LAS-1000; Fuji-Photo Film, Tokyo, Japan). Blots were also probed with the gene for 16 S rRNA as a control.

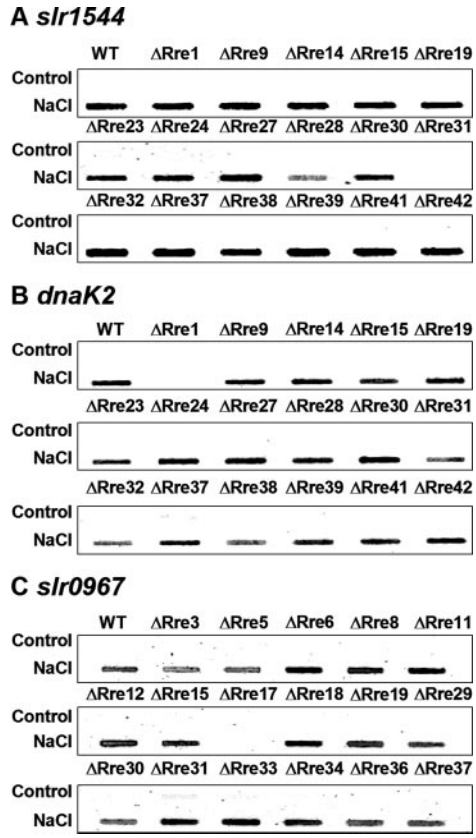

FIG. 1. **Analysis by RNA slot-blot hybridization of the expression of three salt-inducible genes, *slr1544* (*A*), *dnaK2* (*B*), and *slr0967* (*C*).** Total RNA was extracted from wild-type (*WT*) and ΔRre mutant cells before (*Control*) and 20 min after (*NaCl*) treatment with 0.5 M NaCl. 10 μg of total RNA were transferred to the membrane and allowed to hybridize with DNA probes derived from *slr1544*, *dnaK2*, and *slr0967*, respectively, as described under "Experimental Procedures."

DNA Microarray Analysis—Synechocystis DNA microarrays (CyanoCHIP) were purchased from TaKaRa Bio Co. Ltd. (Ohtsu, Japan), and genome-wide analysis of gene expression was performed as described previously (3, 10). All experiments were performed with CyanoCHIP version 1.6, which included 3074 of the 3264 genes on the *Synechocystis* chromosome. Results were quantified with the IMAGENE version 5.5 program (BioDiscovery, El Segundo, CA). Changes in the levels of transcripts of individual genes relative to the total level of mRNA were calculated after normalization by reference to the total intensity of signals from all genes with the exception of genes for rRNAs. The expression of genes in wild-type cells under salt stress was analyzed in four independent experiments. The expression of genes in Hik mutant cells was analyzed in two independent experiments.

Calculations of induction factors and the identification of salt stress-inducible genes were performed as described previously (5). A reference induction factor was calculated for each gene by averaging the induction factors of each respective gene from 22 experiments (four independent experiments with wild-type cells and two independent experiments with each of the ΔHik33, ΔHik34, ΔHik16, ΔHik41, ΔHik10, ΔRre31, ΔRre1, ΔRre17, and ΔRre3 mutants) and used for evaluation of changes in the gene expression in order to avoid variations caused by experimental deviations. The effect of mutation of a Hik or a Rre on gene

expression (RE: effective ratio) was evaluated quantitatively as shown in Equation 1.

$$RE = \frac{(\text{Induction Factor of a mutant sample} - 1.0)}{(\text{Induction Factor of control sample} - 1.0)} \times 100 \qquad \text{(Eq. 1)}$$

In the equation, 1.0 was subtracted from each Induction Factor because the absence of a change in expression corresponds to an Induction Factor of 1.0. We assigned a salt-induced gene whose expression was reduced by mutation of a Hik or Rre when the RE was less than 50. Moreover, we identified a strictly regulated gene when the RE was less than 15. Under these conditions, the extent of induction was considered to be significantly reduced by inactivation of the respective Hik-Rre system.

RESULTS

Slot-blot Screening of the Rre Mutant Library and DNA Microarray Analysis Identified Specific Sets of Salt Stress-inducible Genes under the Control of Rre31, Rre1, Rre17, and Rre3—In prokaryotic signal transduction pathways, a Hik is phosphorylated in response to a stimulus and then the phosphoryl group is transferred to the cognate Rre. After its phosphorylation, the Rre regulates the expression of specific genes (16). Although we demonstrated previously that salt stress due to 0.5 M NaCl is perceived by Hik33, Hik34, and a combination of Hik16 plus Hik41 (5), cognate response regulators of salt sensors have not yet been identified. Therefore, we attempted to identify candidates for Rres that might regulate salt stress-inducible gene expression.

First, we screened a mutant library of Rres (www.kazusa.or.jp/cyanobase/Synechocystis/mutant) by RNA slot-blot hybridization. According to our previous results (5), we selected the following genes for use as probes: the *slr1544* gene, which is regulated by salt stress under the control of Hik33; the *dnaK2* gene, which is under the control of Hik34; and the *slr0967* gene, which is under the control of Hik16 and Hik41. Fig. 1*A* shows some of the results of slot-blot hybridization. Among all the ΔRre mutant cells, only ΔRre31 cells demonstrated the absence of induction by salt stress of the *slr1544* gene. In all the other ΔRre mutant cells, salt induction (induction by 0.5 M NaCl) was similar to that in wild-type cells. These observations suggested that Rre31 might be a candidate for the cognate Rre of Hik33 in the induction by salt stress of the expression of the *slr1544* gene. When the *dnaK2* gene was used as the probe, the absence of salt induction was evident only in ΔRre1 cells in our library of ΔRre mutants (Fig. 1*B*), suggesting that Rre1 might be a candidate for the cognate Rre of Hik34. We performed a similar experiment with *slr0967* as the probe, and the results suggested that Rre17 might be a candidate for the cognate Rre of Hik16 and Hik41 (Fig. 1*C*).

We examined the involvement of the Rre31, Rre1, and Rre17 in transduction of the salt-stress signal by monitoring salt-inducible gene expression using a DNA microarray. Moreover, we postulated that Rre3, which has been shown to be involved in hyperosmotic signal transduction (6), might function as a transducer in the salt-signaling pathway. Therefore, we also examined the effects of mutation of Rre3 on the expression of salt stress-inducible genes.

Table I lists all the salt stress-inducible genes with induction factors higher than 4.0 that were affected (or not) by mutation of Rre31, Rre1, Rre17, and Rre3. The first group of genes, whose induction was diminished in ΔRre31 cells, included the *hliA*, *hliB*, and *hliC* genes for high light-inducible proteins, the *sigD* gene for RNA polymerase σ factor, and seven other genes for proteins of known and unknown function.

The second group of genes, whose induction was reduced in ΔRre1 cells, included the following genes: *hspA* for a small heat-shock protein; *clpB1* for heat-shock protein 100; *dnaK2* for heat-shock protein 70; *pbp* for penicillin-binding protein 4; *dnaJ* for heat-shock protein 40; *sigB* for RNA polymerase σ factor; *sodB* for superoxide dismutase, and eighteen other genes for proteins of known and unknown function. A unique feature of this group is that most of the genes encode heat-shock proteins, all of which are involved in the synthesis and turnover of proteins (17).

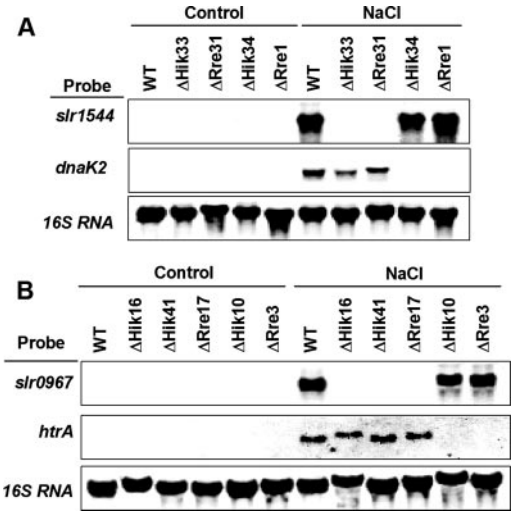

FIG. 2. **Northern blotting analysis of the salt-inducible expression of genes.** *A*, the expression of the *slr1544* and *dnaK2* genes in wild-type (*WT*), ΔHik33, ΔRre31, ΔHik34, and ΔRre1 mutant cells. *B*, the expression of *slr0967* and *htrA* genes in WT, ΔHik16, ΔHik41, ΔRre17, ΔHik10, and ΔRre3 mutant cells. Total RNA was isolated from wild-type, ΔHik mutant, and ΔRre mutant cells before (*Control*) and 20 min after (*NaCl*) treatment with 0.5 M NaCl. The extracted RNA (15 μg) was fractionated on a 1.2% agarose gel that contained 2.05 M formaldehyde and allowed to hybridize with labeled probes derived from *slr1544*, *dnaK2*, *slr0967*, and *htrA* as described under "Experimental Procedures." 16 S rRNA served as a control.

The third group of genes, whose induction of expression was reduced in ΔRre17 cells, included genes for an aspartate transaminase (*sll0938*) and for four proteins of unknown function. The fourth group of genes, whose induction by salt stress was depressed in ΔRre3 cells, was comprised of the *htrA* gene for a presumptive serine protease.

The induction of expression of a group of genes was unaffected by mutation of any of these Rres. This group included the following genes: *ggpS* for glucosylglycerol-phosphate synthase; *glpD* for glycerol-3-phosphate dehydrogenase; *ndhR* for a regulator of transcription of the *ndhF3* operon, and a number of other genes for proteins of known and unknown function.

The results in Table I demonstrate that 42 of 56 salt stress-inducible genes with induction factors higher than 4.0 were under the control of Rre31, Rre1, Rre17, and Rre3. However, 14 of the 56 salt stress-inducible genes were regulated by as yet unknown mechanisms, which might not include an Rre as a component.

DNA Microarray Analysis Identified Hik33, Hik34, Hik16, Hik41, and Hik10 as Upstream Signal Transducers of Rres—Because the quality of DNA microarrays has recently improved considerably, we exploited these improvements to examine in greater detail the effects of mutations in Hik33, Hik34, Hik16, and Hik41 as well as in Hik10, the cognate histidine kinase of Rre3 under hyperosmotic stress (6), on salt stress-inducible gene expression. We compared the profiles of expression of salt stress-inducible genes in Hik mutant cells with those in Rre mutant cells.

The results in Table II demonstrate that the expression of numerous salt stress-inducible genes whose induction by salt stress was controlled by Rre31 was also controlled by Hik33, with the exception of *sll1621*. In the case of ΔHik34 mutant

TABLE I

Salt stress-inducible genes and effects of the inactivation of Rre31, Rre1, Rre17, and Rre3 on the induction of these genes

Cells, grown under control conditions, were incubated with 0.5 M NaCl for 20 min. Each value indicates the ratio of the level of the transcript in salt-stressed cells to that in controls. The numbering of ORFs corresponds to that in the database on the Cyanobase website (ftp.kazusa.or.jp/pub/cyano/Synechocystis/6803ann_new_old3.xls). This table lists the salt stress-inducible genes with induction factors higher than 4.0 in control cells (average of values from 22 independent experiments; see "Experimental Procedures" for full explanation), and their categorization depends on values of RE lower than 50. The entire list can be accessed at www.genome.ad.jp/kegg/expression/.

ORF	Name	Product	Induction by 0.5 M NaCl					
			WT[a]	Δ*Rre31*[b]	Δ*Rre1*[b]	Δ*Rre17*[b]	Δ*Rre3*[b]	*Control*[c]
Genes whose induction by salt stress was reduced in ΔRre31 cells (Group 1)								
slr1544[d]		Putative protein	23.2 ± 0.1	0.8 ± 2.4 (-1)[e]	22.7 ± 5.4 (117)	44.5 ± 0.9 (234)	40.3 ± 3.7 (212)	19.6 ± 3.3
slr1687[f]		Putative protein	16.0 ± 0.4	3.5 ± 0.7 (24)	3.4 ± 0.9 (24)	21.1 ± 0.9 (195)	19.0 ± 0.5 (174)	11.3 ± 1.5
ssr2595	*hliB*	High light-inducible protein	15.1 ± 0.1	0.7 ± 3.4 (-3)	15.8 ± 1.3 (151)	19.5 ± 0.2 (189)	14.4 ± 0.1 (137)	10.8 ± 1.6
ssl2542	*hliA*	High light-inducible protein	9.8 ± 0.0	1.0 ± 2.2 (0)	11.3 ± 4.0 (140)	13.4 ± 2.3 (169)	16.7 ± 0.6 (214)	8.3 ± 1.3
sll1722		Putative protein	10.3 ± 0.2	3.2 ± 6.0 (33)	11.4 ± 0.0 (155)	5.3 ± 0.1 (64)	10.9 ± 2.9 (148)	7.7 ± 1.2
sll1621		Membrane protein	8.4 ± 0.8	3.2 ± 0.1 (36)	5.6 ± 0.2 (74)	8.8 ± 0.7 (127)	8.9 ± 1.2 (128)	7.1 ± 0.5
ssr2016		Putative protein	6.4 ± 0.0	0.8 ± 0.8 (-4)	16.6 ± 2.2 (255)	17.0 ± 1.3 (262)	12.0 ± 0.5 (179)	7.1 ± 1.3
ssl1633	*hliC*	High light-inducible protein	4.9 ± 0.1	1.3 ± 0.3 (7)	12.4 ± 0.7 (229)	11.0 ± 0.4 (201)	8.6 ± 1.4 (153)	6.0 ± 0.9
sll1483		Periplasmic protein	8.0 ± 0.0	0.8 ± 0.6 (-5)	5.9 ± 0.2 (110)	11.2 ± 2.9 (226)	10.7 ± 1.6 (217)	5.5 ± 0.9
sll2012	*sigD*	RNA polymerase σ factor	4.9 ± 0.4	1.8 ± 0.6 (21)	5.6 ± 0.6 (126)	6.8 ± 0.1 (157)	6.8 ± 0.3 (158)	4.7 ± 0.4
sll1797	*ycf21*	Ycf21 gene product	6.2 ± 0.2	2.0 ± 0.4 (30)	5.1 ± 0.1 (122)	6.0 ± 0.3 (148)	6.2 ± 0.9 (155)	4.4 ± 0.5
Genes whose induction by salt stress was reduced in ΔRre1 cells (Group 2)								
sll0528		Putative protein	74.4 ± 4.0	13.6 ± 5.3 (27)	1.5 ± 3.6 (1)	74.6 ± 8.6 (160)	106.9 ± 9.6 (230)	47.1 ± 7.9
sll1514	*hspA*	Small heat-shock protein	49.7 ± 6.8	43.1 ± 1.6 (102)	1.5 ± 0.3 (1)	71.0 ± 1.1 (170)	54.4 ± 6.4 (130)	42.1 ± 5.1
slr0959		Putative protein	19.3 ± 1.1	20.7 ± 0.2 (114)	1.7 ± 0.7 (4)	27.6 ± 3.8 (153)	20.9 ± 2.8 (115)	18.3 ± 2.1
sll0306	*sigB*	RNA polymerase σ factor	20.3 ± 2.2	18.5 ± 1.6 (118)	2.7 ± 0.6 (11)	16.1 ± 1.0 (101)	26.2 ± 7.9 (170)	15.9 ± 1.7
slr1641	*clpB1*	ClpB protein	22.3 ± 1.3	14.8 ± 0.6 (97)	0.8 ± 0.3 (-2)	20.2 ± 1.2 (135)	25.4 ± 4.7 (172)	15.2 ± 1.8
slr1603		Putative protein	22.5 ± 2.1	14.8 ± 2.4 (103)	1.0 ± 0.0 (0)	13.4 ± 2.2 (93)	22.1 ± 2.7 (157)	14.4 ± 1.7
slr0093	*dnaJ*	Heat-shock protein 40	9.4 ± 2.0	17.1 ± 0.0 (138)	0.9 ± 0.7 (-1)	21.3 ± 1.5 (174)	14.3 ± 0.8 (114)	12.7 ± 1.4
sll0846		Putative protein	14.6 ± 1.1	6.9 ± 0.1 (59)	0.9 ± 1.1 (-1)	14.9 ± 4.1 (138)	17.7 ± 2.9 (165)	11.1 ± 1.5
ssl3044		Hydrogenase component	14.5 ± 0.0	6.0 ± 0.2 (51)	2.6 ± 1.5 (16)	17.9 ± 0.2 (171)	14.1 ± 0.3 (133)	10.9 ± 1.2
slr1915		Putative protein	10.3 ± 0.5	14.0 ± 0.1 (143)	0.7 ± 1.1 (-3)	14.1 ± 1.3 (144)	11.9 ± 0.0 (121	10.0 ± 0.9
slr1516	*sodB*	Superoxide dismutase	16.1 ± 2.0	8.9 ± 0.1 (90)	1.9 ± 0.5 (10)	11.6 ± 1.4 (120)	12.5 ± 0.3 (131)	9.8 ± 1.1
sll0170	*dnaK2*	Heat-shock protein 70	12.0 ± 0.2	8.4 ± 0.2 (96)	1.0 ± 0.4 (1)	12.1 ± 2.0 (143)	14.1 ± 2.6 (170)	8.7 ± 1.0
sll1884		Putative protein	11.3 ± 0.8	7.7 ± 0.8 (91)	1.0 ± 0.4 (0)	9.4 ± 0.1 (114)	11.6 ± 0.1 (144)	8.3 ± 0.8
slr1963		Water-soluble carotenoid protein	14.2 ± 2.2	4.5 ± 0.1 (48)	0.8 ± 0.6 (-3)	6.4 ± 0.1 (75)	10.4 ± 0.6 (128)	8.3 ± 1.0
ssr3188		Putative protein	9.4 ± 1.5	6.6 ± 0.2 (86)	1.4 ± 1.4 (7)	9.6 ± 0.8 (132)	9.3 ± 0.6 (128)	7.5 ± 0.6
slr1686		Putative protein	7.3 ± 0.7	6.9 ± 0.1 (103)	1.2 ± 0.5 (4)	10.1 ± 1.3 (160)	6.7 ± 0.5 (100)	6.7 ± 0.7
slr0852		Putative protein	5.9 ± 0.1	9.4 ± 0.2 (161)	0.9 ± 0.6 (-2)	7.4 ± 0.3 (122)	6.9 ± 1.0 (113)	6.2 ± 0.5
sll1167	*pbp*	Penicillin-binding protein 4	11.1 ± 0.5	6.4 ± 0.0 (107)	1.2 ± 0.3 (3)	6.5 ± 0.7 (110)	5.3 ± 1.2 (85)	6.1 ± 0.9
slr0095		*O*-methyltransferase	4.8 ± 0.3	10.9 ± 0.3 (209)	0.8 ± 0.5 (-5)	8.3 ± 0.2 (155)	5.4 ± 0.9 (92)	5.7 ± 0.7
slr1916		Esterase	6.1 ± 0.1	7.1 ± 0.4 (134)	0.8 ± 0.2 (-4)	6.5 ± 0.8 (121)	4.8 ± 0.1 (83)	5.5 ± 0.6
ssl2971		Putative protein	7.2 ± 0.6	5.4 ± 0.4 (103)	1.2 ± 0.3 (4)	6.7 ± 0.8 (134)	6.6 ± 1.1 (131)	5.2 ± 0.5
slr0853	*rimI*	Ribosomal-protein-alanine acetyltransferase	4.5 ± 0.5	7.5 ± 0.0 (165)	1.1 ± 1.4 (2)	5.2 ± 0.1 (106)	4.5 ± 0.9 (90)	4.9 ± 0.5
slr1192		Zinc-containing alcohol dehydrogenase family	5.1 ± 0.2	5.0 ± 0.1 (111)	1.2 ± 0.4 (6)	4.7 ± 0.3 (101)	6.2 ± 1.3 (144)	4.6 ± 0.3
sll0416	*groEL2*	60-kDa chaperonin 2	5.5 ± 0.8	4.5 ± 0.2 (103)	0.6 ± 0.3 (-11)	5.0 ± 0.1 (119)	5.8 ± 0.5 (144)	4.4 ± 0.4
sll1107		Putative protein	5.0 ± 0.3	5.3 ± 0.2 (133)	1.0 ± 0.5 (-1)	4.4 ± 0.7 (103)	5.4 ± 0.3 (136)	4.2 ± 0.3
Genes whose induction by salt stress was reduced in ΔRre17 cells (Group 3)								
sll0939		Putative protein	35.8 ± 3.1	39.2 ± 0.8 (177)	14.1 ± 0.7 (60)	1.2 ± 0.0 (1)	31.2 ± 5.6 (140)	22.6 ± 3.8
slr1704		Putative protein	11.9 ± 3.4	21.6 ± 1.0 (97)	22.6 ± 0.9 (102)	4.5 ± 1.2 (16)	29.6 ± 2.8 (135)	22.2 ± 3.0
slr0967		Putative protein	32.3 ± 8.9	17.3 ± 1.2 (91)	15.4 ± 0.5 (81)	1.9 ± 0.1 (5)	30.3 ± 1.6 (164)	18.8 ± 2.9
ssr2194		Putative protein	8.9 ± 2.1	14.5 ± 9.1 (91)	18.1 ± 7.2 (115)	4.8 ± 1.3 (25)	15.7 ± 0.8 (99)	15.9 ± 2.5
sll0938		*N*-Succinyldiaminopimelate aminotransferase	8.5 ± 4.1	19.3 ± 0.3 (295)	5.5 ± 0.1 (73)	1.1 ± 0.0 (1)	6.2 ± 0.3 (84)	7.2 ± 1.5
Gene whose induction by salt stress was reduced in ΔRre3 cells (Group 4)								
slr1204	*htrA*	Serine protease HtrA	9.5 ± 0.8	8.0 ± 0.1 (144)	5.8 ± 0.0 (82)	6.0 ± 1.0 (102)	1.0 ± 0.0 (0)	5.9 ± 0.7
Genes whose induction by salt stress was unaffected in ΔRre31, ΔRre1, ΔRre17, and ΔRre3 cells (Group 5)								
sll1862		Putative protein	152.4 ± 26.4	121.7 ± 3.3 (80)	142.4 ± 10.1 (93)	211.4 ± 28.6 (139)	228.3 ± 2.9 (150)	152.7 ± 12.6
sll1863		Putative protein	106.5 ± 8.3	141.4 ± 4.1 (115)	110.8 ± 11.1 (90)	160.8 ± 2.5 (131)	133.0 ± 18.3 (108)	122.7 ± 8.1
sll1566	*ggpS*	Glucosylglycerol-phosphate synthase	13.2 ± 2.3	9.6 ± 1.7 (53)	16.1 ± 0.8 (93)	31.0 ± 3.8 (184)	24.6 ± 5.6 (145)	17.2 ± 1.7
sll1085	*glpD*	Glycerol-3-phosphate dehydrogenase	9.7 ± 0.8	8.3 ± 2.6 (70)	11.6 ± 0.2 (102)	18.6 ± 1.2 (169)	14.8 ± 4.3 (133)	11.4 ± 1.0
slr0895		Putative protein	7.2 ± 1.1	11.5 ± 0.5 (16)4	5.9 ± 1.5 (76)	9.1 ± 0.3 (126)	6.0 ± 1.5 (79)	7.4 ± 0.8
sll1652		Putative protein	6.1 ± 0.7	13.1 ± 0.3 (191)	5.3 ± 0.6 (68)	8.6 ± 0.4 (121)	5.7 ± 0.9 (74)	7.3 ± 0.6
sll1594	*ndhR*	Transcriptional regulator of ndhF3 operon	9.7 ± 0.4	7.1 ± 1.2 (101)	8.6 ± 0.8 (126)	5.6 ± 0.4 (77)	6.3 ± 0.6 (88)	7.0 ± 0.5
slr1738		Putative protein	6.5 ± 0.1	5.3 ± 0.8 (83)	3.7 ± 0.5 (53)	7.3 ± 1.0 (122)	7.6 ± 1.3 (128)	6.9 ± 1.0

TABLE I—*continued*

ORF	Name	Product	Induction by 0.5 M NaCl					
			WT[a]	*ΔRre31*[b]	*ΔRre1*[b]	*ΔRre17*[b]	*ΔRre3*[b]	*Control*[c]
ssr2153		Putative protein	4.6 ± 3.5	8.3 ± 0.4 (123)	7.4 ± 3.6 (108)	4.1 ± 0.6 (52)	4.5 ± 0.9 (59)	6.2 ± 0.4
slr1932		Putative protein	5.8 ± 0.2	5.3 ± 1.0 (91)	5.1 ± 0.2 (86)	5.8 ± 0.5 (101)	4.7 ± 0.2 (79)	5.7 ± 0.4
sll1620		Putative protein	6.0 ± 0.4	5.8 ± 0.7 (111)	3.3 ± 0.7 (53)	6.4 ± 1.6 (124)	3.8 ± 0.4 (64)	5.4 ± 0.5
sll1653	*menG*	Probable phylloquinone-biosynthetic methyltransferase	5.1 ± 0.1	7.9 ± 0.5 (163)	3.9 ± 0.5 (69)	6.4 ± 0.2 (128)	4.7 ± 0.0 (89)	5.2 ± 0.4
slr1894		Putative protein	4.9 ± 0.1	3.3 ± 0.2 (53)	3.9 ± 0.0 (114)	6.1 ± 0.8 (57)	6.0 ± 0.2 (84)	4.5 ± 0.5
slr1501		Putative protein	5.2 ± 0.2	2.9 ± 0.7 (67)	5.0 ± 0.0 (84)	3.0 ± 0.3 (150)	4.0 ± 0.4 (146)	4.4 ± 0.3

[a] These values represent the averaged induction factors and ranges of deviation of results, which were calculated from the results of four independent experiments with wild-type cells.

[b] These values represent the averaged induction factors and ranges of deviation of results, which were calculated from the results of two independent experiments with ΔRre mutant cells.

[c] Induction factors and S.D. of controls were calculated by averaging the induction factors from 22 independent experiments (four with wild-type cells and two each with ΔHik33, ΔHik34, ΔHik16, ΔHik41, ΔHik10, ΔRre31, ΔRre1, ΔRre17, and ΔRre3 mutant cells).

[d] Underlining of an ORF indicates a strictly regulated gene whose RE was lower than 15 for some ΔRre.

[e] The number in parentheses is the RE (effective ratio; for definition see "Experimental Procedures").

[f] The *slr1687* gene is categorized as a gene whose inducibility was reduced in ΔRre31 cells (RE = 24), but not in ΔRre1 (RE = 24) cells, because mutation of the cognate Hik33 and Hik34 yielded values of RE of 35 and 54, respectively.

cells, the expression of salt stress-inducible genes under the control of Rre1 was affected by mutation of Hik34. However, the level of expression of several genes, including the *sigB*, *sodB*, and *groEL2* genes as well as that of some genes for proteins of known and unknown function, was reduced in ΔRre1 cells but not in ΔHik34 cells (Table II). These observations suggested that there might be another Hik that perceives salt stress and transfers the signal to Rre1. A similar phenomenon was found in the case of hyperosmotic stress (6). Studies with a yeast two-hybrid system suggested that Hik2 might be the other cognate Hik of Rre1 (6). Therefore, Hik2 might be involved in salt sensing. In the case of the salt stress-inducible genes whose expression was controlled by Rre17, the induction of gene expression was also controlled by Hik16 and Hik41, as shown in Table II. However, there are other genes whose induction by salt stress was reduced in ΔRre17 cells but not in ΔHik16 and ΔHik41 cells. DNA microarray analysis also revealed that Hik10 regulated the expression of the salt stress-inducible gene whose induction was controlled by Rre3 (Table II).

Rescreening of the Rre Mutant Library—The expression of a number of salt-inducible genes was unaffected by mutations in the Rres discussed above. We rescreened the Rre mutant library by slot-blot hybridization using the *ndhR* and *sll1862* genes, which belong to this group of genes, as probes. Our results indicated that the expression of these genes was not abolished in any of the ΔRre mutants (data not shown) and that the expression of the salt-inducible genes in this group might be regulated by some as-yet-unknown mechanism(s).

In *Synechococcus* sp. PCC 7942, a two-component system consisting of NblS and NblR regulates the expression of genes in response to strong light and nutrient stress (18). The homologs of NblS and NblR in *Synechocystis* are Hik33 (18) and Rre28 (as determined by a BLAST search). Therefore, we used DNA microarray analysis to examine the salt-induced expression of genes in ΔRre28 mutant cells. We found that mutation of the *rre28* gene did not affect the expression of salt-inducible genes (data not shown). These observations suggest that Hik33 does not transfer the salt-stress signal to Rre28.

Northern Blotting Analysis of the Effects of Mutations in Rres on the Salt Inducibility of Gene Expression—Our DNA microarray analysis indicated that the salt-inducible expression of the *slr1544* gene was regulated by the Hik33-Rre31 two-component system. We confirmed by Northern blotting the contribution of Hik33 and Rre31 to the induction by salt stress of this gene. Fig. 2 shows that the induction by salt stress of expression of the *slr1544* gene was clearly abolished in both ΔHik33 and ΔRre31 mutant cells. From among the group of salt-inducible genes controlled by Hik34, we selected the *dnaK2* gene to confirm the contributions of Hik34 and Rre1 to salt induction by Northern blotting. Fig. 2 shows that the extent of induction of expression of the *dnaK2* gene was reduced in ΔHik34 and ΔRre1 mutant cells.

We also examined the induction by salt stress of the *slr0976* gene in ΔHik16, ΔHik41, and ΔRre17 cells by Northern blotting. Our results showed clearly that the salt-induced expression of *slr0976* was abolished by mutation of Hik16, Hik41, or Rre17 (Fig. 2). The results of Northern blotting also demonstrated that the extent of induction by salt stress of the *htrA* gene was reduced in ΔHik10 and ΔRre3 mutant cells.

DISCUSSION

Hik-Rre Systems Involved in the Perception and Transduction of Salt Signals in Synechocystis—Our screening of ΔRre and ΔHik mutant libraries by RNA slot-blot hybridization and the genome-wide analysis of gene expression using DNA microarrays indicated that the salt signal is perceived by histidine kinases, such as Hik33, Hik34, Hik16 plus Hik41, and Hik10. Several response regulators, such as Rre31, Rre17, Rre1, and Rre3, receive salt signals from the Hiks and regulate the salt-inducible expression of a very large number of genes. Fig. 3*A* shows a hypothetical model of the salt signal-transducing systems composed of these Hiks and Rres, and it includes the salt-inducible genes that are controlled by the individual systems. Rre1 seems to receive the salt-stress signal from two upstream components. One is Hik34, and the other is likely to be a Hik that constitutes a two-component system with Rre1. Although results with yeast two-hybrid systems suggest that Hik2 might interact structurally with Rre1 (6), there is no direct evidence for any functional interaction between these proteins. Therefore, the nature of the Hik located upstream of Rre1 remains an open question.

In *Escherichia coli*, two two-component systems, namely, EnvZ-OmpR and KdpD-KdpE, are involved in the transduction of osmotic or ionic signals (19–21). Yeast cells utilize a multistep phosphotransfer mechanism that consists of a histidine kinase (Sln1p), a phosphorelay intermediate (Ypd1p), and a response regulator (Ssk1p) when cells are exposed to salt stress (22, 23). In *Synechocystis*, we identified four systems for the transduction of salt signals. Our experiments were successful because we used Rre and Hik mutant libraries in which almost all of the Rres and Hiks had been individually inactivated, as well as genome-wide analysis of gene expression with the DNA microarray. If similar analysis could be applied to other organ-

TABLE II

Effects of the inactivation of Hik33, Hik34, Hik16, Hik41, and Hik10 on the induction by salt stress of gene expression

Cells, grown under control conditions, were incubated with 0.5 M NaCl for 20 min. Each value indicates the ratio of the level of the transcript in salt-stress cells to that in controls. The numbering of ORFs corresponds to that in the database on the Cyanobase website (ftp.kazusa.or.jp/pub/cyano/Synechocystis/6803ann_new_old3.xls). This table lists the salt stress-inducible genes with induction factors higher than 4.0 in control cells (average of values from 22 independent experiments; see "Experimental Procedures" for full explanation), and their categorization depends on values of RE lower than 50. The entire list can be accessed at www.genome.ad.jp/kegg/expression/.

ORF	Name	Product	Induction by 0.5 M NaCl						
			WT[a]	*ΔHik33*[b]	*ΔHik34*[b]	*ΔHik16*[b]	*ΔHik41*[b]	*Hik10*[b]	*Control*[c]
Genes whose induction by salt stress was reduced in ΔRre31 cells (Group 1)									
slr1544[d]		Putative protein	23.2 ± 0.1	1.1 ± 0.1 (0)[e]	7.9 ± 2.4 (37)	25.4 ± 5.4 (131)	14.2 ± 2.5 (71)	10.5 ± 2.0 (51)	19.6 ± 3.3
slr1687		Putative protein	16.0 ± 0.4	4.6 ± 0.4 (35)	6.6 ± 0.7 (54)	12.6 ± 0.9 (112)	9.9 ± 0.0 (86)	10.7 ± 2.4 (94)	11.3 ± 1.5
ssr2595	*hliB*	High light-inducible protein	15.1 ± 0.1	0.9 ± 0.1 (-1)	7.4 ± 3.4 (65)	11.2 ± 1.3 (104)	11.7 ± 1.4 (109)	6.3 ± 2.1 (54)	10.8 ± 1.6
ssl2542	*hliA*	High light-inducible protein	9.8 ± 0.0	0.9 ± 0.0 (-2)	4.7 ± 2.2 (51)	13.9 ± 4.0 (176)	5.0 ± 0.9 (55)	5.3 ± 0.5 (59)	8.3 ± 1.3
sll1722		Putative protein	10.3 ± 0.2	3.4 ± 0.2 (36)	15.2 ± 6.0 (213)	5.9 ± 0.0 (73)	3.2 ± 0.3 (33)	9.7 ± 3.5 (131)	7.7 ± 1.2
sll1621[f]		Membrane protein	8.4 ± 0.8	5.9 ± 0.8 (81)	9.0 ± 0.1 (131)	6.9 ± 0.2 (97)	5.9 ± 0.5 (80)	6.4 ± 1.3 (88)	7.1 ± 0.5
ssr2016		Putative protein	6.4 ± 0.0	0.7 ± 0.0 (-5)	2.1 ± 0.8 (18)	7.1 ± 2.2 (100)	6.4 ± 0.3 (88)	3.2 ± 0.8 (36)	7.1 ± 1.3
ssl1633	*hliC*	High light-inducible protein	4.9 ± 0.1	2.4 ± 0.1 (28)	2.8 ± 0.3 (35)	7.5 ± 0.7 (131)	5.1 ± 1.8 (82)	3.8 ± 1.9 (56)	6.0 ± 0.9
sll1483		Periplasmic protein	8.0 ± 0.0	0.7 ± 0.0 (-7)	2.9 ± 0.6 (41)	4.0 ± 0.2 (67)	2.6 ± 0.3 (35)	5.2 ± 0.8 (94)	5.5 ± 0.9
sll2012	*sigD*	RNA polymerase σ factor	4.9 ± 0.4	1.8 ± 0.4 (23)	3.2 ± 0.6 (59)	6.5 ± 0.6 (149)	4.7 ± 0.5 (101)	4.5 ± 0.8 (95)	4.7 ± 0.4
sll1797	*ycf21*	Ycf21 gene product	6.2 ± 0.2	2.3 ± 0.2 (40)	2.9 ± 0.4 (57)	4.6 ± 0.1 (107)	2.9 ± 0.2 (56)	3.7 ± 0.5 (79)	4.4 ± 0.5
Genes whose induction by salt stress was reduced in ΔRre1 cells (Group 2)									
sll0528		Putative protein	74.4 ± 4.0	16.2 ± 4.0 (33)	22.2 ± 5.3 (46)	47.2 ± 3.6 (100)	35.7 ± 3.6 (75)	45.9 ± 11.6 (97)	47.1 ± 7.9
sll1514	*hspA*	Small heat-shock protein	49.7 ± 6.8	47.2 ± 6.8 (112)	12.1 ± 1.6 (27)	41.8 ± 0.3 (99)	53.0 ± 7.3 (126)	28.3 ± 16.0 (66)	42.1 ± 5.1
slr0959		Putative protein	19.3 ± 1.1	18.2 ± 1.1 (99)	10.0 ± 0.2 (52)	13.1 ± 0.7 (70)	35.2 ± 1.9 (198)	15.5 ± 1.6 (84)	18.3 ± 2.1
sll0306	*sigB*	RNA polymerase σ factor	20.3 ± 2.2	14.5 ± 2.2 (91)	12.5 ± 1.6 (77)	14.3 ± 0.6 (90)	14.4 ± 2.4 (90)	18.9 ± 4.0 (121)	15.9 ± 1.7
slr1641	*clpB1*	ClpB protein	22.3 ± 1.3	9.9 ± 1.3 (62)	7.0 ± 0.6 (42)	18.4 ± 0.3 (123)	11.3 ± 1.3 (72)	22.0 ± 5.9 (148)	15.2 ± 1.8
slr1603		Putative protein	22.5 ± 2.1	11.0 ± 2.1 (74)	9.8 ± 2.4 (66)	18.3 0.0 (129)	8.5 ± 0.1 (56)	18.1 ± 4.0 (128)	14.4 ± 1.7
slr0093	*dnaJ*	Heat-shock protein 40	9.4 ± 2.0	17.2 ± 2.0 (139)	4.5 ± 0.0 (30)	13.1 ± 0.7 (103)	17.9 ± 1.2 (145)	14.5 ± 0.6 (116)	12.7 ± 1.4
sll0846		Putative protein	14.6 ± 1.1	8.5 ± 1.1 (74)	6.5 ± 0.1 (55)	12.2 ± 1.1 (111)	9.9 ± 2.1 (88)	11.8 ± 3.1 (107)	11.1 ± 1.5
ssl3044		Hydrogenase component	14.5 ± 0.0	6.0 ± 0.0 (50)	6.0 ± 0.2 (50)	12.5 ± 1.5 (116)	14.4 ± 2.1 (136)	9.7 ± 1.8 (88)	10.9 ± 1.2
slr1915		Putative protein	10.3 ± 0.5	10.9 ± 0.5 (110)	5.6 ± 0.1 (51)	10.7 ± 1.1 (107	11.9 ± 0.6 (120)	10.6 ± 1.7 (106)	10.0 ± 0.9
slr1516	*sodB*	Superoxide dismutase	16.1 ± 2.0	10.4 ± 2.0 (107	6.4 ± 0.1 (61)	6.7 ± 0.5 (65)	7.7 ± 1.2 (76)	8.3 ± 1.2 (83)	9.8 ± 1.1
sll0170	*dnaK2*	Heat-shock protein 70	12.0 ± 0.2	7.1 ± 0.2 (80)	3.9 ± 0.2 (37)	7.4 ± 0.4 (83)	8.4 ± 1.0 (96)	9.8 ± 1.5 (114)	8.7 ± 1.0
sll1884		Putative protein	11.3 ± 0.8	7.4 ± 0.8 (88)	5.0 ± 0.8 (55)	9.9 ± 0.4 (121)	7.4 ± 0.8 (87)	10.5 ± 0.4 (129)	8.3 ± 0.8
slr1963		Water-soluble carotenoid protein	14.2 ± 2.2	10.5 ± 2.2 (131)	5.1 ± 0.1 (56)	7.0 ± 0.6 (82)	8.6 ± 1.5 (104)	8.9 ± 0.8 (108)	8.3 ± 1.0
ssr3188		Putative protein	9.4 ± 1.5	8.3 ± 1.5 (111)	5.8 ± 0.2 (73)	9.2 ± 1.4 (126)	6.9 ± 0.8 (90)	7.4 ± 1.0 (98)	7.5 ± 0.6
slr1686		Putative protein	7.3 ± 0.7	5.1 ± 0.7 (72)	3.3 ± 0.1 (40)	8.2 ± 0.5 (126)	11.7 ± 0.7 (187)	6.4 ± 1.5 (94)	6.7 ± 0.7
slr0852		Putative protein	5.9 ± 0.1	6.4 ± 0.1 (103)	4.0 ± 0.2 (58)	7.6 ± 0.6 (127)	7.2 ± 0.6 (120)	7.4 ± 1.1 (123)	6.2 ± 0.5
sll1167	*pbp*	Penicillin-binding protein 4	11.1 ± 0.5	6.9 ± 0.5 (117)	0.8 ± 0.0 (-4)	5.8 ± 0.3 (95)	7.0 ± 0.9 (119)	6.2 ± 1.7 (103)	6.1 ± 0.9
slr0095		*O*-methyltransferase	4.8 ± 0.3	7.2 ± 0.3 (131)	2.4 ± 0.3 (29)	5.3 ± 0.5 (92)	8.3 ± 0.3 (155)	5.5 ± 1.1 (95)	5.7 ± 0.7
slr1916		Esterase	6.1 ± 0.1	7.9 ± 0.1 (152)	3.2 ± 0.4 (47)	4.5 ± 0.2 (77)	9.6 ± 1.1 (190)	4.6 ± 0.5 (80)	5.5 ± 0.6
ssl2971		Putative protein	7.2 ± 0.6	4.4 ± 0.6 (81)	3.4 ± 0.4 (58)	5.8 ± 0.3 (113)	3.9 ± 0.1 (70)	6.7 ± 1.1 (136)	5.2 ± 0.5
slr0853	*rimI*	Ribosomal-protein-alanine acetyltransferase	4.5 ± 0.5	6.1 ± 0.5 (129)	3.4 ± 0.0 (61)	6.4 ± 1.4 (138)	7.4 ± 0.9 (162)	6.0 ± 2.1 (127)	4.9 ± 0.5
slr1192		Zinc-containing alcohol dehydrogenase family	5.1 ± 0.2	4.2 ± 0.2 (87)	3.4 ± 0.1 (66)	4.8 ± 0.4 (104)	5.3 ± 0.1 (118)	6.2 ± 0.7 (144)	4.6 ± 0.3
sll0416	*groEL2*	60-kDa chaperonin 2	5.5 ± 0.8	4.9 ± 0.8 (118)	2.8 ± 0.2 (55)	3.6 ± 0.3 (77)	4.6 ± 0.9 (109)	4.4 ± 0.4 (102)	4.4 ± 0.4
sll1107		Putative protein	5.0 ± 0.3	3.6 ± 0.3 (81)	2.9 ± 0.2 (59)	6.1 ± 0.5 (158)	3.7 ± 0.1 (83)	4.5 ± 0.3 (108)	4.2 ± 0.3
Genes whose induction by salt stress was reduced in ΔRre17cells (Group 3)									
sll0939		Putative protein	35.8 ± 3.1	40.8 ± 3.1 (184)	22.8 ± 0.8 (101)	3.1 ± 0.7 (10)	1.2 ± 0.1 (1)	27.6 ± 7.7 (123)	22.6 ± 3.8
slr1704		Putative protein	11.9 ± 3.4	22.0 ± 3.4 (99)	24.7 ± 1.0 (112)	31.8 ± 0.9 (145)	15.6 ± 7.9 (69)	39.4 ± 8.3 (181)	22.2 ± 3.0
slr0967		Putative protein	32.3 ± 8.9	27.4 ± 8.9 (148)	19.5 ± 1.2 (104)	3.5 ± 0.5 (14)	2.0 ± 0.0 (6)	24.9 ± 3.8 (134)	18.8 ± 2.9
ssr2194		Putative protein	8.9 ± 2.1	15.7 ± 2.1 (99)	30.5 ± 9.1 (198)	30.9 ± 7.2 (201)	11.8 ± 5.1 (73)	14.1 ± 8.0 (88)	15.9 ± 2.5
sll0938		*N*-Succinyldiaminopimelate aminotransferase	8.5 ± 4.1	16.5 ± 4.1 (250)	5.8 ± 0.3 (77)	1.0 ± 0.1 (0)	0.9 ± 0.1 (-1)	11.0 ± 4.5 (161)	7.2 ± 1.5
Gene whose induction by salt stress was reduced in ΔRre3 cells (Group 4)									
slr1204	*htrA*	Serine protease HtrA	9.5 ± 0.8	6.4 ± 0.8 (144)	5.0 ± 0.1 (99)	6.8 ± 0.0 (119)	5.4 ± 1.0 (90)	1.2 ± 0.1 (5)	5.9 ± 0.7
Genes whose induction by salt stress was unaffected in ΔRre31, ΔRre1, ΔRre17, and ΔRre3 cells (Group 5)									
sll1862		Putative protein	152.4 ± 26.4	144.3 ± 26.4 (94)	114.5 ± 3.3 (75)	199.3 ± 10.1 (131)	108.1 ± 4.8 (71)	109.2 ± 8.7 (71)	152.7 ± 12.6
sll1863		Putative protein	106.5 ± 8.3	124.9 ± 8.3 (102)	88.8 ± 4.1 (72)	126.4 ± 11.1 (103)	143.8 ± 22.8 (117)	83.8 ± 45.7 (68)	122.7 ± 8.1
sll1566	*ggpS*	Glucosylglycerol-phosphate synthase	13.2 ± 2.3	18.8 ± 2.3 (110)	20.4 ± 1.7 (119)	11.5 ± 0.8 (65)	19.0 ± 2.8 (111)	16.9 ± 3.9 (98)	17.2 ± 1.7
sll1085	*glpD*	Glycerol-3-phosphate dehydrogenase	9.7 ± 0.8	12.6 ± 0.8 (111)	9.1 ± 2.6 (78)	6.5 ± 0.2 (53)	16.2 ± 2.0 (147)	10.9 ± 2.7 (95)	11.4 ± 1.0
slr0895		Putative protein	7.2 ± 1.1	7.3 ± 1.1 (98)	5.3 ± 0.5 (66)	6.4 ± 1.5 (84)	12.3 ± 2.1 (176)	4.0 ± 1.0 (47)	7.4 ± 0.8
sll1652		Putative protein	6.1 ± 0.7	10.0 ± 0.7 (142)	4.0 ± 0.3 (48)	7.2 ± 0.6 (98)	9.4 ± 0.7 (132)	5.5 ± 1.3 (71)	7.3 ± 0.6
sll1594	*ndhR*	Transcriptional regulator of ndhF3 operon	9.7 ± 0.4	5.1 ± 0.4 (69)	7.3 ± 1.2 (105)	5.9 ± 0.8 (82)	5.4 ± 0.1 (74)	4.5 ± 1.6 (59)	7.0 ± 0.5
ssr2153		Putative protein	4.6 ± 3.5	8.0 ± 3.5 (118)	7.6 ± 0.4 (111)	9.4 ± 3.6 (141)	9.4 ± 4.4 (141)	7.4 ± 4.7 (107)	6.9 ± 1.0
slr1738		Putative protein	6.5 ± 0.1	6.4 ± 0.1 (106)	5.2 ± 0.8 (81)	6.5 ± 0.5 (107)	7.0 ± 0.0 (115)	7.0 ± 1.2 (116)	6.2 ± 0.4
slr1932		Putative protein	5.8 ± 0.2	6.7 ± 0.2 (121)	7.0 ± 1.0 (126)	4.5 ± 0.2 (73)	8.8 ± 0.8 (165)	3.7 ± 0.3 (56)	5.7 ± 0.4
sll1620		Putative protein	6.0 ± 0.4	6.4 ± 0.4 (124)	4.8 ± 0.7 (86)	5.1 ± 0.7 (94)	7.0 ± 0.2 (138)	5.4 ± 1.1 (101)	5.4 ± 0.5
sll1653	*menG*	Probable phylloquinone-biosynthetic methyltransferase	5.1 ± 0.1	6.0 ± 0.1 (118)	3.7 ± 0.5 (65)	5.3 ± 0.5 (101)	5.7 ± 1.0 (112)	4.5 ± 0.6 (84)	5.2 ± 0.4
slr1894		Putative protein	4.9 ± 0.1	3.0 ± 0.1 (64)	3.3 ± 0.2 (220)	4.2 ± 0.0 (78)	3.9 ± 0.3 (66)	4.9 ± 0.2 (109)	4.5 ± 0.5
slr1501		Putative protein	5.2 ± 0.2	3.3 ± 0.2 (58)	8.8 ± 2.9 (67)	3.7 ± 0.0 (95)	3.3 ± 0.2 (85)	4.9 ± 0.6 (114)	4.4 ± 0.3

[a] These values represent the averaged induction factors and ranges of deviation of results, which were calculated from the results of four independent experiments with wild-type cells.

[b] These values represent the averaged induction factors and ranges of deviation of results, which were calculated from the results of two independent experiments with ΔRre mutant cells.

[c] Induction factors and S.D. of controls were calculated by averaging the induction factors from 22 independent experiments (four experiments with wild-type cells and two experiments each with ΔHik33, ΔHik34, ΔHik16, ΔHik41, ΔHik10, ΔRre31, ΔRre1, ΔRre17, and ΔRre3 mutant cells).

[d] Underlining of ORFs indicates a strictly regulated gene whose RE was lower than 15 for some ΔRre.

[e] The number in parentheses is the RE (effective ratio; for definition see "Experimental Procedures").

[f] The salt stress-inducible expression of the *sll1621* gene was controlled by Rre31 but not by Hik33.

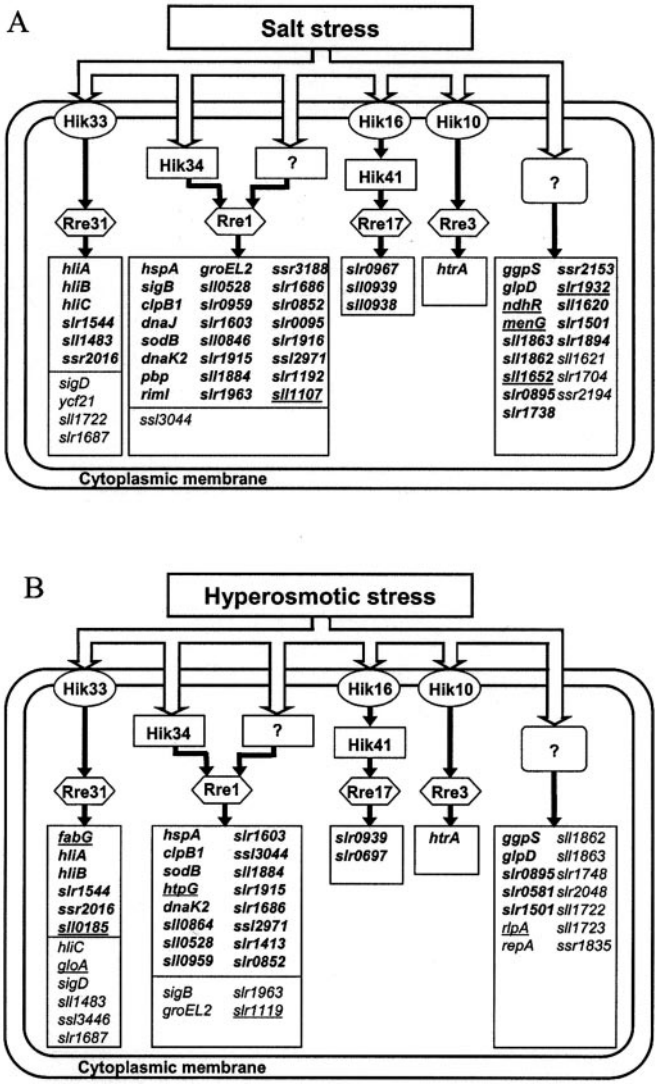

FIG. 3. **Hypothetical scheme for the perception and transduction of salt signals and hyperosmotic signals, including the genes whose salt stress-inducible expression or hyperosmotic stress-inducible expression is regulated by the indicated individual Hik-Rre systems in *Synechocystis*.** Genes included in this scheme revealed induction factors greater than 4.0 under salt stress or hyperosmotic stress. Genes in *bold* letters are genes whose inducibility by salt stress (*A*) or by hyperosmotic stress (*B*) was strictly regulated (RE <15) by a specific Rre, whereas the expression of genes in *light* type was only reduced (RE = 15–50) by the indicated Rres. *Underlining* indicates genes whose expression was specifically induced by salt stress, but not by hyperosmotic stress (the induction factor >4 under salt stress but <2 under hyperosmotic stress (*A*) and genes whose expression was specifically induced by hyperosmotic stress, but not by salt stress (the induction factor >4 under hyperosmotic stress but <2 under salt stress (*B*). The details of the hyperosmotic stress-signaling pathway were generated by recalculation of the DNA microarray results obtained in a previous study (6) according to Equation 1 under "Experimental Procedures."

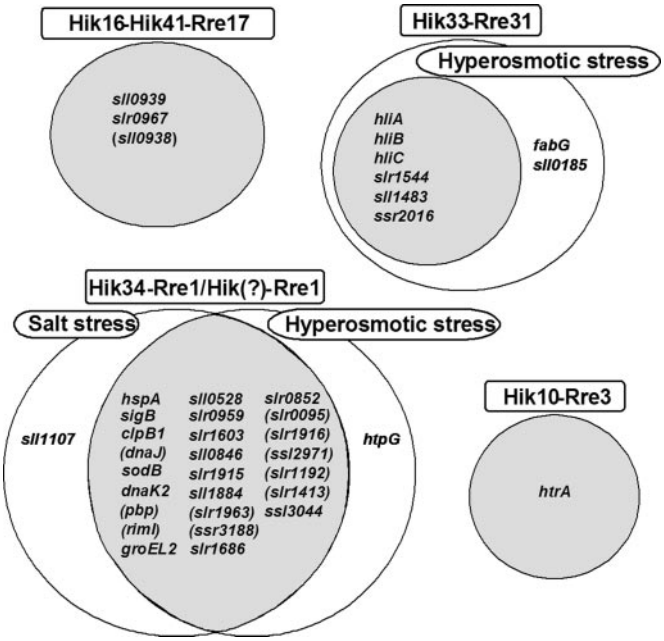

FIG. 4. **Schematic representation of the genes whose expression was strictly regulated (RE <15) by individual Hik-Rre systems under salt stress and/or hyperosmotic stress.** Genes included in this scheme had induction factors >4.0 under salt stress and hyperosmotic stress, with the exception of the genes in parentheses, namely, *sll0938*, *dnaJ*, *pbp*, *rimI*, *slr1963*, *ssr3188*, *slr0095*, *slr1916*, *ssl2971*, *slr1192*, and *slr1413*, which had induction factors between 2.0 and 4.0 under salt stress or hyperosmotic stress. Expression of genes in the *shaded* zones was induced by both salt stress and hyperosmotic stress, whereas the expression of genes in the *white* zones was induced specifically either by salt stress or by hyperosmotic stress as indicated.

isms, such as *E. coli* and yeast, it is likely that many more two-component systems would be identified for the detection and transduction of salt signals.

Identical Sets of Hik-Rre Systems Perceive Salt Stress and Hyperosmotic Stress but Regulate Different Sets of Genes—The Hik33-Rre31, Hik34- or unidentified Hik-Rre1, Hik10-Rre3, and Hik16-Hik41-Rre17 systems appear to function in the perception and transduction of both salt stress and hyperosmotic stress (6). This finding is rather surprising because, as we demonstrated previously, salt-inducible genes differ from hyperosmotic stress-inducible genes (3). Therefore, we compared the sets of salt-inducible genes (Fig. 3*A*) and hyperosmotic stress-inducible genes (Fig. 3*B*) that were under the conspicuous control of individual Hik-Rre systems. Our results led us to propose the scheme presented in Fig. 4.

Fig. 4 shows that in the case of Hik10-Rre3 only the *htrA* gene for serine protease was induced by both kinds of stress. In the case of Hik16-Hik41-Rre17, only three genes, namely, *sll0939*, *slr0967*, and *sll0938*, were induced by both kinds of stress. These schemes are consistent with the currently accepted model of Hik-Rre systems, in which a Hik and its cognate Rre regulate the expression of one gene or a set of genes. The map of genes on the *Synechocystis* genome indicates that *sll0939* and *sll0938* probably form an operon and are, thus, regulated together and that the *slr0967* gene is located just downstream of the operon on the opposite strand of DNA. It seems very likely that the salt-inducible expression of the *sll0939-sll0938* operon and that of the *slr0967* gene are regulated separately.

By contrast, in the case of the three other Hik-Rre systems, Hik33-Rre31, Hik34-Rre1, and unidentified Hik-Rre1, each system regulated the expression of a group of genes that was induced by both kinds of stress and a group of genes whose expression was induced either by salt stress or by hyperosmotic stress. One group of genes, namely *hliA*, *hliB*, *hliC*, and some genes for proteins of unknown function, was controlled by the Hik33-Rre31 system; the expression of these genes was induced by both salt stress and hyperosmotic stress. However, expression of another group that consisted of the *fabG* gene and *sll0185* was induced by hyperosmotic stress, but not by salt stress.

In the case of the Hik34-Rre1 or unidentified Hik-Rre1, the expression of some genes, such as *hspA*, *sigB*, *clpB1*, *dnaK2*, and some other genes for proteins of known and unknown

function, was induced by both salt stress and by hyperosmotic stress. Moreover, this Rre1-mediated system regulated genes whose expression was specifically enhanced by salt stress, but not by hyperosmotic stress, such as the *sll1107* gene. However, this system also regulated genes whose expression was specifically enhanced by hyperosmotic stress, but not by salt stress, such as the *htpG* gene.

Our finding that the two-component systems, Hik33-Rre31 and Hik34-Rre1 or unidentified Hik-Rre1, regulate the expression of distinct respective sets of genes under different kinds of stress cannot be explained by the currently accepted model of two-component systems. For example, although Hik33 might perceive both salt stress and hyperosmotic stress and transduce these signals to Rre31, the model does not explain the differential expression of the two groups of genes under the control of Hik33-Rre31 under different types of stress. The situation for the Hik34-Rre1 or unidentified Hik-Rre1 systems is similar: the differential expression of two groups of genes under different types of stress cannot be explained. It seems reasonable to postulate the presence of some unknown factor(s) that provides each two-component system with strict specificity that is related to the specific nature of the stress. Further studies are necessary to identify these unknown factors and to elucidate how they interact with the various Hik-Rre systems.

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